

WHAT IS CLAIMED IS:

- 1 1. A zinc finger protein that binds to a target site, wherein the target site  
2 has a nucleotide sequence as specified in Table 3 or 4.
- 1 2. The zinc finger protein of claim 1, comprising at least one finger of the  
2 C<sub>2</sub>H<sub>2</sub> class of zinc fingers.
- 1 3. The zinc finger protein according to claim 2, wherein the target site is  
2 one of the nucleotide sequences in a row of Table 3 or 4 and positions -1 to +6 in at least one  
3 of the zinc fingers are occupied by a segment of seven contiguous amino acids as specified in  
4 the row.
- 1 4. The zinc finger protein according to claim 3, wherein positions -1 to  
2 +6 in each of the three zinc fingers are occupied by first, second and third segments of seven  
3 contiguous amino acids as specified in a row of Table 3.
- 1 5. The zinc finger protein according to claim 4, wherein the segments  
2 have the amino acid sequences specified for one of the zinc finger proteins listed in Table 3,  
3 wherein the zinc finger protein is selected from the group consisting of BVO 13A, EP10A,  
4 GATA82Z7678, HBV 3, HP38 4A, HUM 17A, HUM 19A, MTS 5A, MX1E, PDF 5A, RAT  
5 24A, SAN 16A, USX 3A, VEGF 1, VEGF 1\*3, VEGF 1A, VEGF 1B, VEGF 1C, VEGF 1D,  
6 VG 10A, VG 1B, VG 4A, VG 8A, VOP 28A-2, VOP 30A-4, VOP 32A-6, VOP 32B-7, VOP  
7 35A-10, ZEN-7A 1, VOP 29A-3, VOP 32C, VOP 32D, VOP 32E, VOP 32F, VOP 32G,  
8 VOP 32H, VOP 32I and VOP 32J.
- 1 6. The zinc finger protein according to claim 2, wherein the zinc finger  
2 protein comprises six zinc fingers, and positions -1 to +6 in at least one of the six zinc fingers  
3 is occupied by a segment of seven contiguous amino acids as specified in Table 4.
- 1 7. The zinc finger protein according to claim 2, wherein the zinc finger  
2 protein comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are  
3 occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

1                   8.     The zinc finger protein according to claim 7, wherein the segments  
2 have the amino acid sequences specified for a zinc finger protein selected from the group  
3 consisting of BVO 10A-9A, BVO 12A-11B and BVO 14B-13A as listed in Table 4.

1                   9.     The zinc finger protein according to claim 1, wherein the zinc finger  
2 protein is a fusion protein comprising a regulatory domain.

1                   10.    The zinc finger protein according to claim 9, wherein the fusion  
2 protein comprises a plurality of regulatory domains.

1                   11.    The zinc finger protein according to claim 9, wherein the regulatory  
2 domain is an activation domain.

1                   12.    The zinc finger protein according to claim 11, wherein the activation  
2 domain is selected from the group consisting of (a) VP16, (b) p65, and (c) functional  
3 fragments of (a) and (b).

1                   13.    The zinc finger protein according to claim 9, wherein the regulatory  
2 domain is a repressor domain.

1                   14.    The zinc finger protein according to claim 13, wherein the repressor  
2 domain is selected from the group consisting of (a) KRAB, (b) methyl binding domain  
3 protein 2B, (c) v-ErbA repressor domain, and (d) functional fragments of (a), (b) and (c).

1                   15.    A zinc finger protein that binds to a target site having a nucleotide  
2 sequence as specified in Table 3 or 4 whereby the zinc finger protein can modulate  
3 angiogenesis when introduced into an animal having a genome comprising a VEGF gene  
4 comprising the target site.

1                   16.    The zinc finger protein of claim 15, comprising at least three fingers of  
2 the C<sub>2</sub>H<sub>2</sub> class of zinc fingers.

1                   17.    The zinc finger protein according to claim 16, wherein the target site is  
2 one of the nucleotide sequences in a row of Table 3 or 4 and positions -1 to +6 in at least one  
3 of the zinc fingers are occupied by a segment of seven contiguous amino acids as specified in  
4 the row.

1 18. The zinc finger protein according to claim 17, wherein positions -1 to  
2 +6 in each of the three zinc fingers are occupied by first, second and third segments of seven  
3 contiguous amino acids as specified in a row of Table 3.

1 19. The zinc finger protein according to claim 16, wherein the zinc finger  
2 protein comprises six zinc fingers, and positions -1 to +6 in at least one of the six zinc fingers  
3 is occupied by a segment of seven contiguous amino acids as specified in Table 4.

1 20. The zinc finger protein according to claim 19, wherein the zinc finger  
2 protein comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are  
3 occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 21. A nucleic acid encoding a polypeptide, wherein the polypeptide  
2 comprises a zinc finger according to claim 1.

1 22. A nucleic acid encoding a polypeptide, wherein the polypeptide  
2 comprises a zinc finger protein according to claim 4.

1 23. A nucleic acid encoding a polypeptide, wherein the polypeptide  
2 comprises a zinc finger protein according to claim 7.

1 24. A nucleic acid encoding a polypeptide, wherein the polypeptide  
2 comprises a zinc finger protein according to claim 9.

1 25. A method for modulating expression of a VEGF gene, the method  
2 comprising contacting a target site of a nucleic acid within a cell with a zinc finger protein,  
3 wherein the target site has a nucleotide sequence as specified in Table 3 or 4 and binding of  
4 the zinc finger protein to the target site modulates expression of the VEGF gene in the cell.

1 26. The method according claim 25, wherein the expression of a plurality  
2 of splice variants of the VEGF gene is modulated.

1 27. The method according to claim 25, wherein a plurality of target sites  
2 are contacted with a plurality of zinc finger proteins and each zinc finger protein binds to a  
3 distinct target site.

1 28. The method according to claim 27, wherein each of the plurality of  
2 zinc finger proteins is a fusion protein.

1 29. The method according to claim 28, wherein each of the zinc finger  
2 proteins is a fusion protein comprising a regulatory domain.

1 30. The method according to claim 29, wherein each zinc finger protein is  
2 fused to a different regulatory domain.

1 31. The method according to claim 25, wherein the zinc finger protein  
2 comprises at least three fingers of the C<sub>2</sub>H<sub>2</sub> class of zinc fingers.

1 32. The method according to claim 31, wherein positions -1 to +6 in each  
2 of the three zinc fingers are occupied by first, second and third segments of seven contiguous  
3 amino acids as specified in a row of Table 3.

1 33. The method according to claim 31, wherein the zinc finger protein  
2 comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are occupied  
3 by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 34. The method according to claim 25, wherein the zinc finger protein is a  
2 fusion protein comprising a regulatory domain.

1 35. The method according to claim 34, wherein the method further  
2 comprises administering the zinc finger protein in combination with a delivery vehicle.

1 36. The method according to claim 34, wherein the method further  
2 comprises administering a nucleic acid encoding the zinc finger protein into the cell.

1 37. The method according to claim 36, wherein administering comprises  
2 delivering the nucleic acid into the cell in a naked form.

1 38. The method according to claim 36, wherein the nucleic acid is  
2 contained within an expression vector and is operably linked to a promoter, and administering  
3 comprises delivering the vector into the cell.

1 39. The method according to claim 38, wherein the expression vector is a  
2 viral expression vector.

1 40. The method according to claim 39, wherein the expression vector is  
2 selected from the group consisting of a retroviral expression vector, an adenoviral expression  
3 vector, and an AAV expression vector.

1 41. The method according to claim 38, wherein the promoter is an  
2 inducible promoter.

1 42. The method according to claim 34, wherein regulatory domain  
2 comprises an activation domain and binding of the zinc finger protein to the target site  
3 activates transcription of the VEGF gene in the cell.

1 43. The method according to claim 42, wherein the cell is a population of  
2 cells.

1 44. The method according to claim 43, wherein activation of VEGF  
2 transcription activates angiogenesis in the population of cells.

1 45. The method according to claim 44, wherein the population of cells is a  
2 cell culture.

1 46. The method according to claim 44, wherein the population of cells are  
2 in a mammalian subject.

1 47. The method according to claim 36, wherein the zinc finger protein or  
2 zinc finger protein nucleic acid are administered in an amount effective to treat a disease or  
3 injury.

1 48. The method according to claim 47, wherein the disease or injury is  
2 selected from the group consisting of atherosclerosis, ischemia and arthritis.

1 49. The method according to claim 47, wherein the subject has a wound  
2 and the amount administered is effective to treat the wound.

1 50. The method according to claim 47, wherein the subject has an ulcer  
2 and the amount administered is effective to treat the ulcer.

1 51. The method according to claim 42, wherein activation of VEGF  
2 transcription activates lymphogenesis in the population of cells.

1                    52.    The method according to claim 42, wherein activation of VEGF  
2 transcription activates myelopoiesis in the population of cells.

1                    53.    The method according to claim 42, wherein the activation domain is  
2 selected from the group consisting of (a) VP16, (b) p65, (c) functional fragments of (a) and  
3 (b).

1                    54.    The method according to claim 34, wherein the regulatory domain is a  
2 repressor domain and binding of the zinc finger protein to the target site represses  
3 transcription of the VEGF gene in the cell.

1                    55.    The method according to claim 54, wherein the cell is a population of  
2 cells.

1                    56.    The method according to claim 55, wherein repression of VEGF  
2 transcription represses angiogenesis in the population of cells.

1                    57.    The method according to claim 55, wherein the population of cells is a  
2 cell culture.

1                    58.    The method according to claim 55, wherein the population of cells are  
2 in a mammalian subject.

1                    59.    The method according to claim 58, wherein the zinc finger protein or  
2 zinc finger protein nucleic acid are administered in an amount effective to treat a disease or  
3 injury.

1                    60.    The method according to claim 59, wherein the disease is a tumor.

1                    61.    The method according to claim 54, wherein the repressor domain is  
2 selected from the group consisting (a) KRAB, (b) methyl binding domain protein 2B, (c) v-  
3 ErbA repressor domain, and (d) functional fragments of (a), (b) and (c).

1                    62.    The method according to claim 25, wherein the target site is located in  
2 a single type of VEGF gene, and binding of the zinc finger protein to the target site modulates  
3 expression of the single VEGF gene in the cell.

1           63.     The method according to claim 25, wherein the target site is located in  
2 a plurality of different types of VEGF genes, and binding of the zinc finger protein to the  
3 target site modulates expression of the plurality of VEGF genes.

1           64.     The method according to claim 63, wherein the target site comprises a  
2 nucleotide sequence bound by a protein selected from the group consisting of EP10A,  
3 GATA82Z678, HBV 3, HP38 4A, HUM 17A, MTS 5A, PDF 5A, USX 3A, VEGF 1,  
4 VEGF1\*3, VEGF 1A, VG 10A, VG 1B, VG 4A, VG8A, VOP28A-2, VOP 30A-4, and ZEN-  
5 7A 1.

1           65.     The method according to claim 64, wherein the target site is the  
2 nucleotide sequence recognized by VOP 28A-2.

1           66.     The method of according to claim 64, wherein the target site is the  
2 nucleotide sequence recognized by VOP 30A-4.

1           67.     A method for modulating angiogenesis comprising introducing a zinc  
2 finger protein into an animal having a genome comprising a target site within a VEGF gene,  
3 whereby the zinc finger protein binds to the target site and thereby modulates angiogenesis in  
4 the animal.

1           68.     The method according to claim 67, wherein the zinc finger protein  
2 binds to a target site specified in Table 3 or 4.

1           69.     The method according to claim 68, wherein positions -1 to +6 in each  
2 of three zinc fingers are occupied by first, second and third segments of seven contiguous  
3 amino acids as specified in a row of Table 3.

1           70.     The method according to claim 68, wherein the zinc finger protein  
2 comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are occupied  
3 by a segment of seven contiguous amino acids as specified in a row of Table 4.

1           71.     The method according to claim 67, wherein the target site is present in  
2 a plurality of VEGF genes, whereby the zinc finger protein binds to the target site in the  
3 plurality of genes, thereby modulating expression of the plurality of VEGF genes.

1           72.    The method according to claim 67, wherein introducing comprises  
2 introducing a plurality of zinc finger proteins into the animal, each zinc finger protein binding  
3 to a different target site in the same gene.

1           73.    The method according to claim 72, wherein each of the zinc finger  
2 proteins is a fusion protein comprising a regulatory domain.

1           74.    The method according to claim 73, wherein each zinc finger protein is  
2 fused to a different regulatory domain.

1           75.    A method of treating ischemia, comprising administering a zinc finger  
2 protein that binds to a target site specified in Table 3 or 4 into an animal having ischemia,  
3 wherein the zinc finger protein is administered in an amount effective to treat ischemia.

1           76.    The method of claim 75, wherein the animal has a genome comprising  
2 a VEGF gene comprising the target site and the zinc finger protein binds to the target site.

1           77.    The method according to claim 76, wherein the zinc finger protein  
2 comprises at least three fingers of the C<sub>2</sub>H<sub>2</sub> class of zinc fingers.

1           78.    The method according to claim 77, wherein positions -1 to +6 in each  
2 of the three zinc fingers are occupied by first, second and third segments of seven contiguous  
3 amino acids as specified in a row of Table 3.

1           79.    The method according to claim 77, wherein the zinc finger protein  
2 comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are occupied  
3 by a segment of seven contiguous amino acids as specified in a row of Table 4.

1           80.    A method for screening for a modulator of expression of a VEGF gene,  
2 the method comprising:

3           (a)    contacting a test cell with a zinc finger protein and a test agent,  
4 wherein the zinc finger protein comprises at least one zinc finger that binds to a target site,  
5 the target site having a nucleotide sequence as specified in Table 3 or 4;

6           (b)    comparing the level of expression of the VEGF gene in the test cell  
7 with a baseline level, a statistically significant difference in the level of expression in the test

cell relative to the baseline level indicating that the test agent is a potential modulator of VEGF gene expression.

81. The method of claim 80, wherein the zinc finger is a fusion protein comprising an activation domain, and a lower level of expression in the test cell relative to the baseline level indicates that the test agent is a repressor of the VEGF gene.

82. The method of claim 80, wherein the zinc finger protein is a fusion protein comprising a repressor domain, and an increased level of expression in the test cell relative to the baseline level indicates that the test agent is an activator of the VEGF gene.

83. A pharmaceutical composition comprising a nucleic acid according to claim 14 operably linked to a regulatory sequence and a pharmaceutically acceptable carrier or diluent, wherein the regulatory sequence allows for expression of the nucleic acid in a cell.

84. The pharmaceutical composition according to claim 83, wherein the nucleic acid is contained in an expression vector.

85. The pharmaceutical composition according to claim 84, wherein the expression vector is a viral expression vector.

86. The pharmaceutical composition according to claim 85, wherein the expression vector is selected from the group consisting of a retroviral expression vector, an adenoviral expression vector, and an AAV expression vector.

87. A pharmaceutical composition comprising a zinc finger protein according to claim 1 and a pharmaceutically acceptable carrier or diluent.

88. A zinc finger protein comprising a plurality of zinc fingers, wherein at least one of the plurality of zinc fingers is occupied by a segment of seven contiguous amino acids as specified in a row of Table 3 or 4.

89. The zinc finger protein of claim 88, wherein the zinc finger protein is a three finger zinc finger protein and the at least one zinc finger is occupied by a segment of seven contiguous amino acids as specified in a row of Table 3.

1 90. The zinc finger protein of claim 89, wherein at least two of the zinc  
2 fingers are occupied by a segment of seven contiguous amino acids as specified in a row of  
3 Table 3.

1 91. The zinc finger protein of claim 90, wherein all three of the zinc  
2 fingers are occupied by a segment of seven contiguous amino acids as specified in a row of  
3 Table 3.

1 92. The zinc finger protein of claim 88, wherein the zinc finger protein is a  
2 six finger zinc finger protein and the at least one zinc finger is occupied by a segment of  
3 seven contiguous amino acids as specified in a row of Table 4.

1 93. The zinc finger protein of claim 92, wherein at least three of the zinc  
2 fingers are occupied by a segment of seven contiguous amino acids as specified in a row of  
3 Table 4.

1 94. The zinc finger protein of claim 93, wherein all six of the zinc fingers  
2 are occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 95. A method for treating a wound comprising introducing a zinc finger  
2 protein into an animal having a genome comprising a target site within a VEGF gene,  
3 whereby the zinc finger protein binds to the target site, such binding accelerating healing of  
4 the wound.